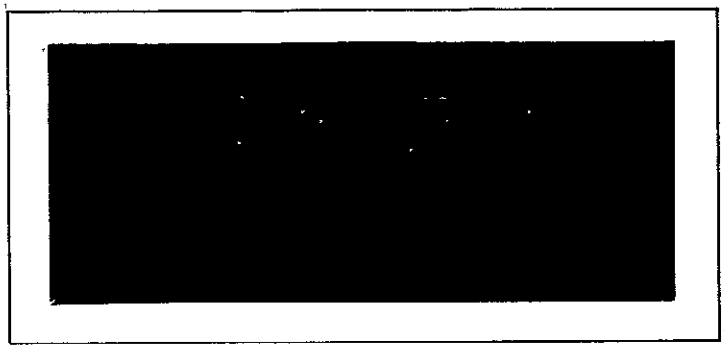
DRA



(NASA-CR-148529) TECHNOLOGY FOR RETURN OF PLANETARY SAMPLES Quarterly Progress Report, 19 Aug. - 31 Oct. 1975 (Biospherics, Inc.) 69 p HC $4.50 CSCL 03B

N76-29138

Unclas

G3/91 15155

BIOSPHERICS INCORPORATED

4928 WYACONDA ROAD, ROCKVILLE, MARYLAND 20852 • TELEPHONE (301) 770-7700

Report

"TECHNOLOGY FOR RETURN OF PLANETARY SAMPLES"

Quarterly Progress Report
19 August 1975 - 31 October 1975

Prepared for:

National Aeronautics and Space Administration
NASA Headquarters
Washington, D. C. 20546

Prepared by:

Biospherics Incorporated
4928 Wyaconda Road
Rockville, Maryland 20852

December 18, 1975

BIOSPHERICS INCORPORATED

TABLE OF CONTENTS

BIOSPHERICS INCORPORATED

LIST OF TABLES

BIOSPHERICS INCORPORATED

LIST OF FIGURES

BIOSPHERICS INCORPORATED

I. SUMMARY

The purpose of this contract is to provide NASA with assistance in planning the return of a Mars sample to Earth. Emphasis has been placed on the development of a basic warning system to assess the biohazard of a Mars return sample or spacecraft prior to its return to Earth. Further, design concepts are under investigation for the development of a Planetary Receiving Laboratory that will meet the scientific requirements of investigators while maintaining quarantine conditions.

In the development of a basic warning system, the model ecosystem concept has been advanced as the most reliable, sensitive method for assessing the biohazard from the Mars sample before it is permitted on Earth. Two approaches to ecosystem development have been studied. In the first approach, the Mars sample would be introduced into the ecosystem and exposed to test conditions which are as similar to the Martian environment as the constitutent terrestrial organisms can tolerate and still function. This approach gives the Martian organism the best chance of survival and permits it the maximum expression of its capabilities. Consequently, the literature has been surveyed to select the most hardy terrestrial candidates. Laboratory-stressed microorganisms as well as organisms which are naturally subjected to harsh terrestrial environmental conditions, namely, alpine tundras, desert biomes, and the dry valleys of Antarctica have been reviewed.

In the second approach, the Mars sample would be tested to determine its effects on important terrestrial cellular functions. This might be accomplished with the ecosystem under either Mars or terrestrial conditions or, preferably, in separate ecosystems under each set of environmental conditions. Consequently, a survey of key cellular functions has been conducted to delineate those most important to terrestrial life for testing in the model ecosystem.

BIOSPHERICS INCORPORATED

Further, the possibility of combining these two approaches by examining the effect of the Mars organism on these vital processes in terrestrial organisms which can function at or near Mars-like conditions has been examined.

In addition to the development of a basic warning system, efforts have been directed toward establishing design considerations for the Mars Planetary Receiving Laboratory. The problems encountered with the Lunar Receiving Laboratory have been evaluated in this context. A questionnaire has been developed to obtain the necessary requirements from knowledgeable scientists for important experiments to be conducted in the Planetary Receiving Laboratory.

Efforts for the next reporting period will proceed with selection and the beginning of laboratory evaluation of some simple candidate ecosystems. The Planetary Receiving Laboratory study will also be pursued with questionnaire distribution and assessment of initial returns therefrom.

BIOSPHERICS INCORPORATED

II. DEVELOPMENT OF THE ECOSYSTEM CONCEPT AS A BASIC WARNING SYSTEM

A. Rationale

One of the options for the next step in planetary exploration, perhaps in the 1980's, is the return of material from Mars (1). Our present state of knowledge requires that any Martian sample must be regarded as a possible source of life forms dangerous to Earth. Considerable experience with the problems of maintaining quarantine of extraterrestrial material was gained during the Apollo program. Despite precautions such as the Lunar Receiving Laboratory and Quarantine Facility, the Earth was contaminated with lunar material beginning with the re-entry of Apollo craft into the atmosphere and continuing at intervals throughout the quarantine period (2). Quarantine procedures for Martian samples should be more rigorous not only because of past experience but also because prospects for life on Mars are greater than they were for the moon.

One objective of the Mars sample return program is to design an improved Planetary Receiving Laboratory (PRL) for absolute containment. However, some possibility always exists that the quarantine will be broken. The Space Science Board concluded (3) that "extraterrestrial life and the concomitent possibility of back contamination must be presumed to exist," and an AIBS study group (4) concluded that "current LRL quarantine protocol is by no means adequate to protect the Earth from contamination by injurious agents of extraterrestrial origin." Some of the possible adverse effects of extraterrestrial life on Earth life have been enumerated (5, 6, 7).

These concepts, coupled with the fact that there is no way to ascertain absolutely that Martian organisms are not pathogenic or that they cannot adversely affect some vital process on Earth, have led to considerable opposition (2) to the return of a Mars sample to Earth

regardless of quarantine precautions. In a survey (8) of prominent scientists, several possible methods were offered to minimize the risk. Dr. J. Lederberg of the Stanford Medical School suggested that tests be conducted on Mars to determine whether terrestrial environments were inhibitory for growth of Martian organisms. Dr. Klein of the Ames Research Center suggested that experiments exposing a variety of terrestrial organisms or delicately balanced ecosystems to Martian soil be conducted on Mars prior to the return of a sample to Earth. Dr. M. Alexander of Cornell University concluded (8) that a determination of the species essential to the Earth's biosphere should be made and that selected representative species should be included in a quarantined exposure to the Mars sample. In an elaboration of these studies (7), Alexander suggested monitoring for possible modifications in key microbial reaction sequences such as photosynthesis, CO_2 evolution, O_2 consumption, nutrient regeneration, and nitrogen fixation. Population abundance and species diversity could also be studied.

In agreement with the ecosystem approach, one of the specific recommendations of the AIBS Board (4) was that "the most significant test system may be that of Martian material exposed to the widest possible spectrum of Earth conditions involving at least several Earth ecosystems... The approach could be made by beginning with simple soil systems with associated microfauna and flora, and, in later tests, extended up the food chain to higher plants and animals." The use of test systems such as bacterial cultures or small mouse colonies has been suggested by others (9).

Thus, although no direct determination of pathogenicity on man can be made, an assessment of the effect of a planetary sample on vital Earth processes may be achieved through using model ecosystems as a Basic Warning System. These tests may be performed in Mars orbit, during the return flight, on the moon, or in Earth orbit. In this way

a decision may be made regarding the disposition of the sample before the Earth's atmosphere has been contaminated. The development of such a system and its testing prior to allowing any samples to be brought to Earth may provide the needed safeguard and, also, a means for preventing "...adverse public reaction to a returned, unsterilized sample as a result of concern for damage to life" (1, 10). This ecosystem can monitor biohazards resulting from interference with the basic terrestrial life processes. If any vital process is interfered with, the changes to our system will be clearly indicated. While a negative result cannot rule out some adverse effect on Earth, it would lend considerable assurance that a catastrophic impact is unlikely should some Mars material escape to our environment.

B. Types of Candidate Systems

An ecosystem may be defined as "...the total of interacting physicochemical and biological factors in a microenvironment" (11). This is a nonrestrictive definition including forests at one extreme and a laboratory slant culture at the other. Some definitions do restrict the term "ecosystem" to environments functioning in nature. Our ecosystem of necessity must be removed from nature.

There are several reasons for choosing as simple a system as possible. First, a multi-level ecosystem is difficult to maintain under the best of laboratory conditions. Second, a balanced complex ecosystem will not readily respond to alteration in a single species as it is not sensitive to small changes (12). Third, even if a response does occur, it will be difficult to monitor because of compensation by other species moving into the vacant niche. Thus, the choice of a simple model ecosystem is predicated on the need for a sensitive system that will show a definite quantifiable response to an adverse effect caused by a Martian life form.

BIOSPHERICS INCORPORATED

The main adverse effects of a Mars life form on terrestrial life are two-fold. First, the Mars organism may produce disease in man or the plants and animals upon which he depends for survival. Second, the Mars organism may interfere with some biological process vital for the continuance of life, such as photosynthesis or nitrogen-fixation. A model ecosystem which permitted direct observation of higher life forms over long periods of time would assess the possibility of disease production, while a microbial system can be used to determine effects on the vital processes represented. Other potential effects, such as the consumption or transformation of some necessary resources, would be difficult to evaluate in model ecosystems.

One of the main problems in developing a model ecosystem is the selection of the organisms. These organisms may be macrobiota such as animals or plants, or microbiota such as protozoa, fungi, algae, or bacteria. The problems encountered with a macrobiotic ecosystem for use away from Earth are centered around the need for extensive life support systems for these organisms to survive the journey to Mars. This system would lend itself more readily to testing in an orbiting laboratory or in the Planetary Receiving Laboratory. A microbiotic system can be maintained by a lower level of life support and, to illustrate the extreme, may survive in a lyophilized form. Therefore, an ecosystem containing microorganisms can be more readily sustained for long periods of space flight. In this report we have concentrated on developing a microbial ecosystem which can be transported to the vicinity of Mars to serve as a biohazard warning device.

The organisms that will compose this microbial ecosystem can be selected either because they survive well under a particular set of environmental conditions or because they represent a key metabolic pathway which needs to be investigated. In the first approach, the Martian organisms would be exposed to one set of test conditions

which are as similar to the Martian environment as the constituent terrestrial organisms can tolerate and still function. These conditions would give any Martian organisms the best chance of multiplying and permit the maximum opportunity for expression of potentially destructive capabilities.

The second approach addresses the specific processes to be studied in these ecosystems. It is important that the basic cellular functions and nutrient cycle reactions be challenged by a Martian organisms. This approach concerns the selection of representative organisms for studying these key processes. Tests under terrestrial conditions would permit optimal baseline functioning of the Earth organisms and thus make any adverse effect on the process studied easier to detect and quantify. Information on the inhibitory effects of the terrestrial environment may become apparent by comparison of this terrestrial system with the Mars-like system.

The possibility of combining these two approaches will be considered. In such an approach, the effect of the Mars organisms on one or more terrestrial vital processes, as exemplified by Earth organisms selected for hardiness at or near Mars conditions, would be studied. The final selection of ecosystem type and organisms will depend on extensive laboratory testing to ensure a sensitive and reliable indicator system, to determine its ability to withstand the prolonged journey to the point of use, and to be compatible with existing equipment limitations for life support and monitoring.

In preparation for the selection of ecosystems consistent with each approach, a literature review has been conducted. For the first approach, the literature on laboratory stressed microorganisms and organisms in harsh terrestrial environments has been surveyed to select the most hardy candidates (Section II. B. 1.). For the second, an analysis of basic cellular functions and the microbial participation

in nutrient cycles has been carried out to select candidate representatives of these vital processes (Section II. B. 2.).

1. Microbial Candidates for a Martian Ecosystem

Under this concept, the organism(s) for the Martian ecosystem will be selected on the basis of their ability to function in a Mars-like environment. Several sources of information are pertinent to this selection. First, various studies have exposed terrestrial microorganisms to Mars-like stresses. Second, studies of harsh environmental extremes on Earth, namely Arctic tundras, desert biomes, and dry valleys of Antarctica will be examined to determine which terrestrial organisms can best function under harsh environmental conditions.

a. Environmental Conditions on Mars

Much of the current data about Mars was gained from the Mariner 9 mission. Atmospheric pressure at the surface was reported to vary from 2.8 millibars in the Cloritas and Tharsis areas to 10.3 millibars near the north polar cap. The mean atmospheric surface pressure was 5.2 ± 1.0 millibars with a seasonal variation of ± 1.5 (13). The surface temperature ranged from -128°C to +27°C with a diurnal freeze-thaw cycle in some regions. The diurnal variation is believed to range up to as much as 100 centigrade degrees (14). The absence of an ozone shield layer in the atmosphere indicates that the full ultraviolet radiation from the sun reaches the surface of Mars although there may be some shielding from dust particles.

One of the key considerations for terrestrial growth is the amount of available liquid water. While the question of whether liquid water is present on Mars has not been absolutely resolved, indications are strong that little or none is available.

The atmospheric content of water vapor averages in the range of 10-20 precipitable microns (13); but, seasonally, the thin atmosphere

may approach saturation. The prime source of the vapor is thought to be the polar caps which, although primarily composed of solid carbon dioxide, contain appreciable water-ice (14). Subsurface juvenile water, permafrost and volcanic activity are other sources of water vapor. The problem with respect to life is that the temperature and pressure conditions on most, if not all, of Mars are such that water may never exist in the liquid phase. If so, it is possible the Martian organisms may have evolved to absorb water vapor and conserve condensed water internally for use in an aqueous biochemistry. Barring this assumption, however, Mars life forms based on an aqueous biochemistry would be dependent on the availability of liquid water in their environment. Some mechanisms have been proposed by which liquid water may become available diurnally or for brief periods. First, some depressions on Mars are sufficiently deep to provide the atmospheric pressure necessary to support liquid water within a narrow temperature range. Formation of nighttime condensate retained by adsorption on steep sloping surfaces facing away from the sun (15), transfer from the atmosphere by adsorption on settling dust (13) and the formation of liquid water under the pressurized condition of a "greenhouse effect" produced beneath ice surfaces (C. B. Farmer, personal communication) are other possible mechanisms.

The Martian atmosphere consists almost entirely of CO_2, although argon and trace amounts of CO, O_2, and O_3 have been detected in addition to the water vapor. Of more serious consequence to life forms is that nitrogen is present in only trace quantities, if at all (14). Obviously, conditions on Mars are harsh by terrestrial standards.

b. Laboratory Studies Under Mars-like Stresses

Although natural conditions on Earth do not approach the severity of Mars, laboratory studies have been conducted to determine the survival of organisms under "Mars-like" conditions. In the beginning

phase of research on survival in Mars-type environments, emphasis was placed on the development of Mars chambers. Although we know that these earlier experiments did not truly stimulate Martian conditions, particularly with respect to the availability of liquid water, they have provided information (16) on the tolerance of organisms to a number of stresses that would be experienced on Mars.

In studies (17) under reduced atmospheric pressure, cocci and actinomycetes have been found to be among the most resistant types, suggesting an ability to survive the atmospheric conditions to which a Martian form would be accustomed. In a more rigorous study (18), the organisms Bacillus mesentericus, B. mycoides, B. megaterium, B. simples, Actinomyces globisporus, Aspergillus oryzae and A. terreus were found to have 100% survival rates after exposure to a vacuum of 10^{-8} to 10^{-10} mm Hg. Since a variety of microorganisms have been found to be resistant to reduced atmospheric pressure, the Mars atmospheric pressure is not considered a limiting factor for the survival of terrestrial microorganisms.

One of the more limiting factors for life would be the ultraviolet radiation reaching the surface of Mars. Much work has been done on the effects of UV radiation on bacterial cells generated by the interest in the enzymatic repair reactions (19), and in the use of ultraviolet radiation as a method of sterilization (20, 21). Various mechanisms, including shielding by dust, growth under particles, and evolved pigmentation, have been postulated as defense mechanisms that might protect an organism from the effect of UV under Martian conditions. The cocci, Micrococcus radiodurans and Sarcina lutea, and the spores of Bacillus subtilis, have been found to be among the most resistant organisms with 700,000, 1970 and 1000 ergs/cm^2 required, respectively, to inhibit colony formation in 90% of the cells (22). However, among bacteria, resistance may differ even among strains of the same species. In the algae Chlorella, the

photosynthetic system was found (23) to regenerate 70% of capacity after complete suppression of activity by UV radiation. However, available data (20, 21, 22) indicate that exposure to the full effect of the ultraviolet radiation reaching the surface of Mars would be lethal to most terrestrial organisms. Hence, the amount of ultraviolet radiation will probably have to be reduced to an acceptable level, or sufficient shielding provided for the terrestrial organisms selected for the ecosystem.

Another serious restriction is the required ability to survive repeated cycles of freezing and thawing. The problem is not so much being able to withstand a particular high or low temperature, but rather surviving the repeated cycling between extremes outside the normal range of growth temperatures. That most microorganisms will survive freezing is demonstrated by the well accepted preservation method of lyophilization. However, the process of freezing and thawing is quite detrimental to natural populations as noted in the reduced plate counts obtained from frozen samples compared to those from unfrozen controls (24, 25). Survival under conditions of diurnal temperature cycles, usually in the range of $-70^{\circ}C$ to $+25^{\circ}C$, have been studied in Mars chambers. Frequently test conditions include atmospheres high in N_2 and low in CO_2. Most included relatively large amounts of water by Mars standards. Scher, et al. (26) found that obligate and facultative anaerobic spore formers and some facultative anaerobic nonspore formers did survive, but did not grow, under conditions which included 10^{-9} erg/cm^2 ultraviolet radiation and $CaSO_4$ to remove water. There are many reports (27, 28, 29) of spore formers, notably B. cereus and B. subtilus, surviving and growing under diurnal freeze-thaw cycles when a sufficient amount of moisture (6-8%) was present. If the moisture level were reduced to 4%, the spore would germinate; but the resulting cells would not be viable (30). Hawrylewicz, et al. (31) also determined that one effect

of the freeze-thaw cycle was that growth of spores could occur at a soil moisture concentration which would normally limit growth of the cells at 35°C. This work was performed under terrestrial atmospheric conditions and in a nutrient medium.

In further work, Young, et al. (32,33) confirmed that spore formers have relatively little resistance to freeze-thaw cycles unless the thaw duration exceeds four and one-half to eight hours. A consistent, but low, rate of killing permitted survival of A. aerogenes, but complete and rapid killing occurred in other genera, notably, Spirillum, Rhodopseudomonas and Pseudomonas (34,35). Although most of the work has been on procaryotic organisms, the protozoan Calpoda maupasii has been extensively studied (36,37). Cyst stages were better able to survive freeze-thaw cycles than vegetative cells, even in cycles from 22-24°C to -196°C under conditions of slow cooling. In Colorado, some areas experience as many as 238 freeze-thaw cycles per year (38). In summary, research in this area has shown that some organisms can survive repeated freeze-thaw cycles if the thaw cycle is of sufficient length to permit cell multiplication. However, a moisture concentration far in excess of that on Mars was found to be necessary for growth of the organisms tested.

Other factors to be considered are the concentration of oxygen and the available nutrients. The trace amounts of atmospheric oxygen on Mars would not be limiting to all candidate terrestrial microorganisms since facultative or obligate anaerobes, capable of growing under anaerobic conditions, can be selected. The problem of available nutrients, however, is not readily resolved. At this time, little is known about the composition of Mars "soils," and test situations involving heterotrophic organisms will, therefore, probably require nutrient supplements.

Consideration of the above studies points to the key probable inhibitory factors for terrestrial microorganisms in a Martian

environment. Hence, resistance to these stresses is a prime criterion in the choice of candidate terrestrial organisms for the test ecosystem. Many terrestrial microorganisms survive reduced atmospheric pressure, some can survive variations in temperature, and forms exist which do not require oxygen. Terrestrial microorganisms probably cannot withstand exposure to Martian ultraviolet radiation although some forms are more tolerant than others. Consequently, the level of ultraviolet irradiation in the test ecosystem should be reduced, or shielding should be provided. Mars moisture levels appear to be the most severely limiting condition for terrestrial microorganisms. Some experiments have demonstrated an interaction between moisture and temperature cycles with regard to their effect on microorganisms. A balance between the level of moisture and the temperature cycle will have to be established to permit survival and metabolism of candidate terrestrial microorganisms.

c. Organisms Inhabiting Harsh Terrestrial Environments

Although no area on Earth is subjected to all the extremes of the Mars environment, there are some terrestrial environments in which one or more environmental parameters is extreme. Information is available about the abilities of microorganisms to survive in areas of low temperature (Arctic tundras, dry Antarctic valleys) and extreme dryness (deserts, dry Antarctic valleys) particularly through research programs performed in conjunction with the International Biological Program (IBP) from 1968 to 1973. Examination of these environments in the following sections will assist in the selection of organisms best adapted to such harsh conditions.

(1) Arctic Tundras

Arctic tundras exemplify the effects of extreme cold temperatures. Tundras generally have short growing seasons, and are wet and cold with soils deficient in minerals. Much of the United States

research (39) has concentrated on the Barrow, Alaska coastal tundra, an area underlain by perennially frozen soil ranging from 20-40 cm to over 300 m deep (40). Clarholm, et al. (41) have found bacteria in all the tundra soil samples examined. Pseudomonas has been identified as the main form in wet tundra sites and Bacillus as the main form in dry areas. Parinkina (42) noted that the bacterial numbers fluctuated markedly among samples taken over a few days' time. As would be expected, greater numbers of organisms were found in areas supporting vegetation than in mineral soils (43).

Much of the emphasis in the IBP tundra program has been on the nitrogen cycle since area soils are particularly poor in nitrates, but are not carbon limited (44). The principal inorganic form of nitrogen is ammonia resulting from N_2 fixation (45). In wet tundra areas, the blue-green algae, Nostoc and Anabaena, are responsible for most of the nitrogen fixed (46). In the dry tundras, few algae or leguminous plants are found, and lichens, such as Nephroma or Peltigera are the primary agents of nitrogen fixation (47, 48). The bacterial contribution to the nitrogen fixation budget is not as significant as that of lichens or algae (49).

The main agents of photosynthesis in both wet and dry tundras are lichens and plants. All of the plant species tested demonstrated positive photosynthesis at temperatures as low as 0-5°C (50). In addition, blue-green algae photosynthesize in the wet tundras but do not predominate in dry areas although some algae, notably Chlamydononas nivalis, form blooms on hard frozen surfaces (51). Lichens found in both wet and dry areas are predominantly Nephroma, Peltigera and Collema (46). Thus, lichens are the main microbial agents of primary productivity in extremely cold, dry areas.

The environment in arctic tundras is so extreme that many of the species have adapted to these areas. For instance, greater concentra-

tions of mobile elements, such as potassium and phosphorus, have been found in tundra species than in comparative temperate forms (52). In summary, research in tundra areas indicates that lichens, which are organisms adapted to cold, dry tundra regions, should be considered for the Mars-like ecosystem. Furthermore, since these organisms are capable of both photosynthesis and nitrogen-fixation, they may be candidates for ecosystems combining both Mars-like conditions and emphasizing vital processes.

(2) Deserts

In deserts, the effects of moisture, which is a limiting factor for most microbial growth therein, can be studied to assist in the selection of microorganisms which may tolerate the extreme aridity of the Martian environment. In addition, deserts are generally subjected to extremely high temperatures during the day followed by cold nights. Biologically available water is usually scarce in temperate or tropical deserts, and then suddenly comes in deluges or in unavailable forms, such as snow or ice in colder climates. Furthermore, since only 10% of the solar radiation may be deflected, compared to 60% deflection in more humid regions (53), organisms in these areas are exposed to higher amounts of ultraviolet radiation.

These varied conditions are reflected in the widely differing bacterial counts that have been reported in deserts. Numbers of bacteria vary from as few as 10 cells/g by the plate count method, to as many as 6.3 to 8.4×10^7 cells/g by the direct counting method (17). Numbers of bacteria in arid or semi-arid soils receiving 25 to 35 cm of annual precipitation have been found to approach 1.0×10^6 cells/g soil (54) by the plate count method. These variations may primarily reflect the differences in the available water. Even in naturally moist soils, the addition of water increases biological respiration (55). Both heterotrophic and chemoautotrophic types of bacteria were isolated with sulfur metabolizers in the majority (56).

Unlike the tundra areas, which are nitrogen limited, deserts are carbon limited. Plants are specifically adapted to hot, dry climates. During drought conditions, the metabolism of these plants slows, as evidenced by a reduction in carbon dioxide exchange (44). Algae and lichens are present as hard crusts or concentrated under translucent or transparent materials (56). Such materials probably enhance growth by permitting light transmission and providing moisture through condensation on their undersides by means of the greenhouse effect (the mechanism advanced by Farmer for Mars). Cameron and Blank (56) have extensively studied these forms and list 16 common species among which are Anacystis marina, Nostoc muscorum, and Chlorella vulgaris. Nitrogen is fixed rapidly in the deserts (44) with rates of 10-20 g/m^2 per year noted. These rates were attributed to fixation by blue-green algae in the soil crusts. Thus, because these algae and lichens have survived under conditions of extreme aridity, as typified by desert climates, they should be considered as possible candidates for the Mars-like ecosystem.

(3) Dry Valleys of Antarctica

The dry valleys of Antarctica encompass extremes of both cold and dryness (57). Because both conditions are present, these areas are considered to be the most harsh terrestrial environments and the most similar to Mars. The valleys are ice-free, but the mean annual temperature is $-20^{\circ}C$ to $-25^{\circ}C$. Diurnal freeze-thaw cycles can occur with the surface of the ground reaching $15^{\circ}C$, or so, for short periods. The water vapor content is an order of magnitude less than that in more temperate areas and the soil is both saline and alkaline. Generally, it is the lack of moisture that is the limiting factor in these regions. The loose water content (that driven off by heating at $110^{\circ}C$ for 24 hours in vacuo) ranges from 3.6 to 18.1 mg/g soil. This is several orders of magnitude lower than the sun baked temperate

zone soils (57). Upon the addition of moisture to these soils, the bacterial population may increase from three to six orders of magnitude (58).

Only heterotrophic organisms, bacteria and fungi, have been identified in these areas of extreme cold and aridity. Generally, the bacteria are aerobic, heterotrophic, mesophilic nonsporulating rods or cocci. The lack of prevalence of sporeformers is noteworthy (58). Coryneforms and arthrobacters were the most prevalent types, but Bacillus, Brevibacterium and Micrococcus sp. have also been found. Neither Pseudomonas nor Cytophaga species were detected (59). Enrichments for chemoautotrophic organisms such as Thiobacillus and Nitrosomonas have yielded several isolates, but these have not yet been identified (60). At least ten genera of filamentous fungi have been found with the majority of species belonging to the genera Cladosporium and Penicillium. The yeasts, Tilletiopsis and Sporobolmyces, have been identified (60) as well.

Other microbial species, both blue-green and coccoid algae, yeasts, molds and protozoa have been found in areas of higher moisture, and in lakes, in Antarctica. Although common in other areas of Antarctica, lichens have not been reported in the dry valleys (58). These findings suggest that heterotrophic organisms, primarily bacteria, may best be able to survive under extreme multiple stresses. The predominant types, coryneforms and arthrobacters, should be considered candidate hardy organisms.

d. Candidate Organisms Based on Survival Under Harsh Conditions

Data from the laboratory studies and ecological surveys have been summarized in Table 1. For each stress, organisms are listed alphabetically within broad taxonomic groups: bacteria, blue-green algae, eucaryotic algae, lichens, molds and yeasts. Information on the response of some microorganisms to a particular

Table 1

Summary of the Data Indicating the Ability of Various Groups of Microorganisms to Survive Harsh Environmental Conditions

Stress	Martian Conditions	Organisms	Response to Stress
1. Reduced Atmospheric Pressure	2.8 mb to 10.3 mb		1. 10^{-8} mb Atmospheric Pressure
		Actinomyces	Resistant
		Bacillus	Resistant
		Clostridium	Resistant
		Escherichia coli	Sensitive
		Klebsiella	Resistant
		Micrococcus aurantiacus	Resistant
		Micrococcus luteus	Resistant
		Mycobacterium	Resistant
		Pseudomonas	Sensitive
		Sarcina	Resistant
		Serratia	Resistant
		Staphylococcus aureus	Resistant
		Vibrio	Sensitive
		Aspergillus	Resistant
2. Ultraviolet Radiation	2000 Ergs/cm^2 (calculated) (Ref. 61)		2. Ergs/cm^2 (2537A°) Required to Kill 90% of Cells
		Bacillus anthracis	452
		B. megaterium (cells)	113
		B. megaterium (spores)	273
		B. subtilis (spores)	1200
		Corynebacterium diphtherium	337
		Escherichia coli	300
		Micrococcus candidus	605
		Proteus vulgaris	264
		Pseudomonas aeruginosa	550
		Pseudomonas fluorescens	350
		Salmonella typhimurium	800
		Sarcina lutea	1970
		Serratia marcescens	242
		Staphylococcus albus	184
		Streptococcus lactis	615
		Aspergillus niger	2200
		Penicillium roqueforti	216
		Saccharomyces	133
		Micrococcus radiodurans	700,000 Ergs/cm^2, wavelength unspecified

Table 1
(continued)

Stress	Martian Conditions	Organisms	Response to Stress
3a. Reduced Temperature (Natural Environment)	-128°C min.		3a. -20°C to +15°C
		Actinomyces	Resistant
		Arthrobacter	Resistant
		Bacillus	Resistant
		Brevibacterium	Resistant
		Corynebacterium	Resistant
		Micrococcus	Resistant
		Blue-Green Algae (Unspecified)	Resistant
		Lichens (Unspecified)	Resistant
		Aspergillus	Resistant
		Cladosporium	Resistant
		Penicillium	Resistant
		Sporobolomyces	Resistant
		Tilletiopsis	Resistant
3b. Diurnal Temperature Cycle (Laboratory)	-128°C to + 25°C		3b. -70°C to + 25°C
		Aerobacter aerogenes	Resistant
		Bacillus (8% Moisture)	Resistant
		Bacillus (<4% moisture)	Sensitive
		Pseudomonas	Sensitive
		Rhodopseudomonas	Sensitive
		Spirillum	Sensitive
		Staphylococcus aureus	Resistant
4. Minimal Moisture	Extact Amount Unknown		4. Moisture Conditions
		Actinomyces	Desert
		Arthrobacter	0.48% to 1.7% (wt.)
		Brevibacterium	0.48% to 1.7% (wt.)
		Clostridium (Spores)	Laboratory Tests
		Corynebacterium	0.48% to 1.7% (wt.)
		Micrococcus	0.48% to 1.7% (wt.)
		Mycobacterium	Desert
		Radiobacter	Desert
		Streptomyces	Desert
		Chlamydononas	Dry Tundra
		Chlorella	Desert
		Microcoleus	Desert
		Nostoc	Desert
		Phytoconis	Desert
		Porphyrosiphon	Desert

Table 1
(continued)

Stress	Martian Conditions	Organisms	Response to Stress
		Protococcus	Desert
		Protosiphon	Desert
		Schizothrix	Desert
		Scytonema	Desert
		Collema	Dry Tundra
		Nephroma	Dry Tundra
		Peltigera	Dry Tundra
		Gladosporium	Dry Valleys
		Penicillium	Dry Valleys
		Sporobolmyces	Dry Valleys
		Tilletiopsis	Dry Valleys
5. Oxygen	Trace Amounts		Requirement for Oxygen
		Obligately Aerobic Bacteria	+
		Obligately Anaerobic Bacteria	-
		Facultatively Anaerobic Bacteria	-
		Blue-Green Algae	-
		Eucaryotic Algae	-
		Lichens	?
		Molds	+
		Yeasts	-
6. Nutrients	Exact Composition Unknown	...	Organic Carbon Requirement
		Heterotrophic Microorganisms	+
		Autotrophic Microorganisms	-, May Need Vitamins

stress is not available since not all strains have been laboratory tested for all parameters. Further, ecological studies frequently emphasized the group responsible for a particular process but did not identify individual genera or species, for example, the nitrogen-fixing blue-green algae.

These data demonstrate that, while the response to some stresses, such as the trace amounts of oxygen, is characteristic of a taxonomic group, response to others, such as ultraviolet radiation, cannot be categorized and may differ significantly among strains of the same species.

Let us first consider those stresses which will completely eliminate some groups from further consideration as candidates for survival under Mars-like conditions. The trace amounts of oxygen in the Martian atmosphere indicate that organisms with an absolute requirement for oxygen, the obligately aerobic heterotrophic and autotrophic bacteria, and the molds, will probably not survive exposure to Mars-like atmospheric conditions unless oxygen depletion mechanisms or anaerobic microenvironments operate with the test system. The latter do, of course, operate on Earth, but such a system would be difficult to establish in a small test enclosure. However, the facultatively and obligately anaerobic bacteria, the blue-green algae, eucaryotic algae and yeasts can grow in the absence of oxygen. Whether the fungal component of a lichen can survive on the oxygen produced by the partner algae needs to be demonstrated.

Another restrictive condition is the potential scarcity of nutrients which suggests that autotrophic organisms would survive better than heterotrophs. All heterotrophic organisms will require some small addition of nutrients. Even the photoautotrophic microorganisms may require an addition in the form of vitamins, nitrogen or a reducing source which may be water, sulfur or an organic reductant while the chemoautotrophs would require hydrogen, nitrate, or, possibly, sulfur.

BIOSPHERICS INCORPORATED

Therefore, supplementary compounds may be required for any terrestrial microorganisms. The final determination of a nutrient addition would depend upon soil composition information from the current Viking series and the requirements of the test organisms chosen. On the basis of this criterion, minimal available nutrients, all the fastidious organisms should be eliminated from consideration. Organisms known to survive in areas of minimal nutrients, such as tundras and deserts, would be good candidates, as are autotrophic microorganisms.

An interrelationship between moisture and temperature cycling has been noted. An increase in the moisture level increases the survival of some strains. Whether the additional water functions as a protective mechanism against temperature changes, or simply decreases stress, is not known. The environmental studies have provided candidates that can survive low temperatures and low moisture concentrations.

In considering the bacteria found in these categories, the elimination of those with a strong requirement for oxygen leaves the genera: Actinomyces, Corynebacterium, and Clostridium. In laboratory study, the Clostridium species have required high moisture levels to survive temperature cycling, and should not be considered further unless an 8% moisture level is found to be a requirement for the other strains as well. Since Actinomyces and Corynebacterium are known to survive low temperatures, low moisture levels, low nutrient concentrations, and do not require oxygen, they are good bacterial candidates.

A variety of photosynthetic organisms has been found in desert and Arctic environments and species are listed in Table 1. The ability of these organisms to grow without an organic carbon source, their growth under conditions of low temperature and low moisture make them good candidates. Of these organisms, the blue-green algae, Nostoc and the green algae, Chlorella, are extensively used in laboratory studies.

However, these organisms do present several problems. First, oxygen is produced as a result of their photosynthetic activities and may build to toxic levels. Secondly, they require water because their photosynthetic systems depend on photolysis of water. Photosynthetic bacteria may offer advantages because they neither need nor produce oxygen; and they substitute compounds, such as sulfur or organic reductants, for water in the photosynthetic process. Methods for removing or reducing the evolved oxygen should be investigated to determine whether algae should be candidates.

Fungi, as a general group, have been eliminated because of their oxygen requirements. However, fungal utilization of oxygen may be one method of removing the oxygen produced by the algal lichen component. Lichens have the reputation of hardiness and tolerance to cold, aridity and low nutrient conditions, demonstrated by their survival in tundras and deserts. Certainly Nephroma or Peltigera are hardy candidates. Several species of yeast were also found in the dry Antarctic valleys, demonstrating their ability to withstand cold, aridity and low nutrient levels.

However, a more definitive choice of organisms depends on several factors. One is the ability to survive under multiple stresses. Another is that the organism must not only survive under these conditions, but must also actively metabolize if an effect is to be detected. All of these organisms listed have survived in harsh environments. However, these stresses may be relieved periodically by environmental changes or anomalies, and it has been suggested that it is under these circumstances that the majority of the growth occurs. For example, the algae found in deserts are frequently under objects which will permit moisture accumulation (56). Snow algae, such as Raphidonema, survive where liquid water would seem to be biologically unavailable. However, the cells find moisture in "meltwaters" located below the

surface (51). Lichens survive in conditions of great aridity, the deserts. However, studies (62) have shown that lichens in California deserts grow on moisture provided by marine fogs. Furthermore, it has been suggested that lichens grow only under high moisture conditions (63) and that they are not found in the dry valleys of Antarctica because of the unmitigated low moisture levels (58). Consequently, the ability of all of these candidate organisms to metabolize under low moisture conditions needs to be evaluated. Also, the strains most resistant to ultraviolet irradiation and reduced atmospheric pressure need to be determined. These are areas where laboratory studies are required.

2. Candidates Representative of Vital Processes

An alternative approach to selecting the microbial ecosystem is to choose organisms highly representative of basic life processes to be challenged by the Martian life form. Greater assurance of the continued survival of terrestrial life is gained from survival of the test system if it represents vital processes common to all levels of Earth life. These basic processes are of two types. One operates at the cellular level and consists of metabolic reactions embodied, for example, in the amino acid and carbohydrate pathways. The other type operates at the environmental level and consists of ecological nutrient cycles, such as the carbon cycle and the nitrogen cycle.

Life on Earth uses carbon as a molecular backbone, while energy is stored in certain phosphate and sulfate bonds. The continued ability of a terrestrial microorganisms to utilize a carbon containing compound in the presence of a Martian life form could demonstrate, among other things, that cell permeability, transport mechanisms, meatbolic pathways, energy systems, DNA transcription, the molecular mechanisms of enzyme substrate reactions and the necessary trace elements are not destroyed or inhibited. In an extended experiment, information concerning duplication of genetic material and cell growth

and division become available. Thus, from the continued survival and metabolism of a single-celled organisms, assurance could be gained that the basic cellular metabolic and genetic functions are not inhibited by a Martian life form.

At the environmental level, microorganisms are responsible for the cyclic transformations which provide elements and compounds, such as oxygen and nitrate, for cellular metabolism. Some of the reactions in these cycles involving organic carbon compounds, sulfate, and phosphate are common to the majority of organisms and are studied as part of the basic cellular metabolism. Other reactions such as the ability to fix nitrogen, are restricted to certain organisms and specific representatives may need to be considered. Therefore, both cellular metabolic processes and ecological cycles will be considered to determine which organisms can be used to study the continued functions of key vital processes in a model ecosystem.

a. Cellular Functions

Generally, metabolic processes are divided into catabolic, or degradative, pathways and anabolic, or synthetic pathways. These two types of pathways join in the following three areas:

(1) Carbon Sources

The metabolism of most foods will eventually yield a small group of product molecules. These are triose phosphates and pyruvate for carbohydrates; acetyl CoA, propionyl CoA, and glycerol for fatty acids; and acetyl CoA, oxaloacetate, α-ketoglutarate, fumarate and succinate for proteins. In the Tricarboxylic Acid Cycle (Figure 1) these catabolic products are converted to the intermediates used in anabolic pathways for the synthesis of amino acids, purines, pyrimidines and long-chain fatty acids.

BIOSPHERICS INCORPORATED

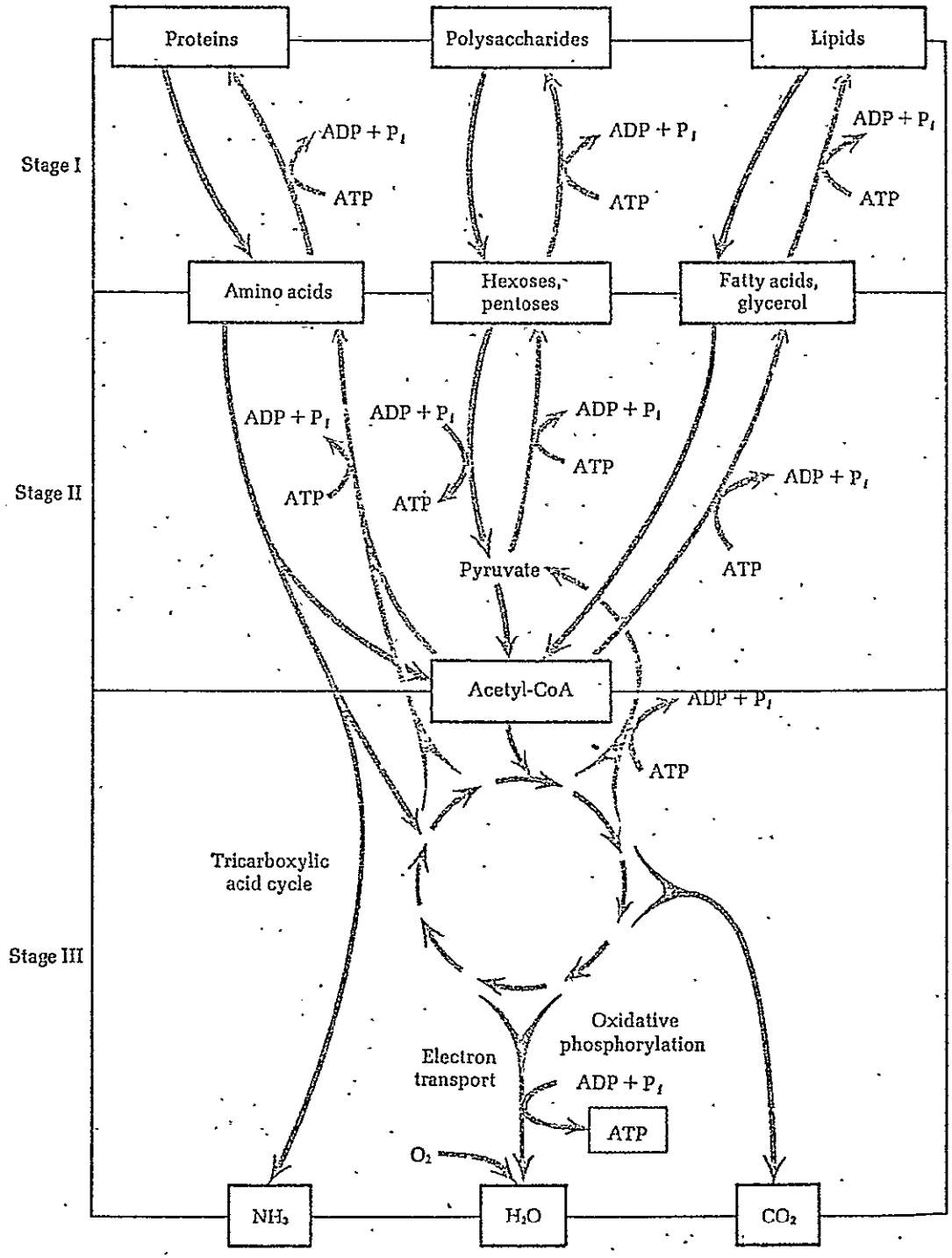


Figure 1

The Interaction of the Tricarboxylic Acid Cycle in the Conversion of Nutrients to Carbon Dioxide, Water, and Energy (64)

(2) Energy Generation

The catabolic processes produce energy in the form of ATP which is utilized in the anabolic synthetic reactions.

(3) Red-Ox Reactions

The reductants generated during catabolism, generally $NADH_2$, provide a source of reducing power for the anabolic processes. It is through the interaction of the Tricarboxylic Acid Cycle and the Electron Transport System that carbon compounds are completely combusted to carbon dioxide and water with the concomitent release of energy in the form of ATP and the production of reducing power in the form of $NADH_2$ (65).

These three areas are interrelated through the Tricarboxylic Acid Cycle and the Electron Transport System. Therefore, the continued functioning of reactions necessary for maintenance and growth in aerobic organisms can be evaluated by metabolism of carbon compounds through those pathways. An additional advantage is that most of the ^{14}C-labeled compounds currently being utilized in the "labeled release" life detection experiment aboard the Viking Mars lander (66) can be metabolized by these pathways. The choice of organisms in which to study these processes is not restricted to obligate aerobes, since facultative anaerobes will preferentially use the Tricarboxylic Acid Cycle and the Electron Transport System under aerobic conditions because of the greater energy yield (67). Therefore, there are many organisms which are capable of aerobic heterotrophic growth from which candidates can be chosen (68).

b. Ecological Nutrient Cycles

The effects of Martian life on ecological processes may best be studied in terms of the elements or compounds necessary

for life namely, carbon, oxygen, water, nitrogen, phosphorus and sulfur. These are readily recognized as participants in the following cycles of matter (Figure 2):

(1) Carbon Cycle

The carbon cycle can be considered in terms of the fixation of carbon dioxide into organic matter through the process of photosynthesis and its subsequent return to the atmosphere through the metabolism and respiration of microorganisms, plants, animals and humans. The process of organic carbon metabolism and the evolution of CO_2 through respiration are general cellular functions and were considered in the previous section. Photosynthesis, the process whereby carbon dioxide is fixed into organic compounds, can be studied in certain organisms only.

The main photosynthetic agents on land are the seed plants with the algae being minor contributors. In the oceans, however, free-floating marine algae are most productive with the larger algae, the seaweeds, being restricted to a narrow coastal zone. Estimates of relative photosynthetic activity (70, 71) suggest that the marine environment is the site of from 40 to 80% of the total primary productivity.

Although marine algae are the main agents of photosynthesis, they require a saline environment which might be detrimental to the Mars organisms and, at this time, are not considered candidate organisms for this reason. The conditions chosen to study these vital processes should not be such that the Martian organism would be subjected to the extremes of terrestrial environment. The emphasis should be on the effect of the Martian organism on the vital terrestrial biological processes and not on the effect of terrestrial conditions on the Martian organism. The saline problem with marine algae can be circumvented by using blue-green algae which are widely distributed in nature and occur not

BIOSPHERICS INCORPORATED

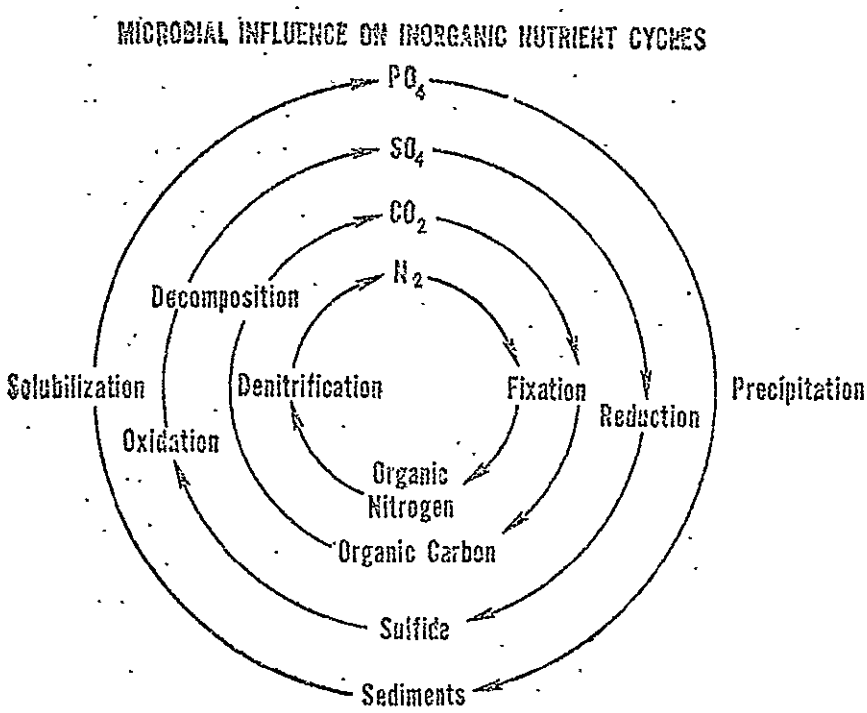


Figure 2

Influence of Microorganisms in the Four Major Nutrient Cycles (69)

only in salt and fresh waters, but also in soils. Furthermore, green algae and blue-green algae have basically the same photosynthetic systems.

The bacterial photosynthetic system, however, differs in several respects from the algal and plant systems. The absence in bacteria of the Type II reaction center, where photolysis of water occurs, necessitates use of an exogenous reductant such as H_2S or an organic compound. This means that oxygen is not produced. Moreover, bacterial photosynthesis is an anaerobic process, carried out by a select group of organisms, the Rhodospirillaceae, the Chromatiaceae, and the Chlorobiaceae. Much information (72) is available about bacterial photosynthesis and its general processes are basically similar. Certainly bacterial representatives of photosynthesis should be considered. It is suggested that blue-green algae should also be included because they carry out an oxygen productive type of photosynthesis, are ubiquitous in nature, and have been well studied in laboratory and field experiments.

(2) Nitrogen Cycle

Most of the Earth's nitrogen occurs occluded in primary rocks as dinitrogen (73), or as the inert gas, N_2, in the atmosphere. The cyclic transformation of nitrogenous compounds is of great importance because while all organisms require nitrogen, not all of them can fix atmospheric nitrogen. The majority of organisms require nitrogen in a combined form such as ammonia, nitrate, nitrite, or incorporated into organic compounds such as amino acids.

Simplistically, the nitrogen cycle consists of the following steps. The fixation of atmospheric nitrogen into a reduced form is carried out by leguminous plants and select free-living procaryotic microorganisms. Decomposition of the organic matter and subsequent release of nitrogen as ammonia, or ammonification, is microbial in nature. The ammonia

produced can be utilized by a wide variety of plants and microorganisms for growth, or it may be modified to nitrite and nitrate in the process of nitrification. The primary agents in this process are the chemolithotrophs such as Nitrosomonas and Nitrobacter. Nitrate can then be used by plants and microorganisms for growth or may serve as the terminal electron acceptor in the process of bacterial denitrification. Tne enzymes required for denitrification are widespread among microorganisms appearing in such diverse genera as Neurospora, Proteus, Hemophilus, Rhizobium, Achromobacter, Spirillum and Corynebacterium. Abiologic transformations, both natural, such as ozonation and lightning-fixation, and industrial, such as the Haber process, also contribute to the nitrogen cycle.

To some degree, the process of ammonification and denitrification can be studied as part of normal cell metabolism since these abilities are widespread in the microbial world. Nitrification is a far more restricted, but less necessary, process than nitrogen fixation. In studies (11) where specific inhibition of ammonia oxidation has occurred for three months, it was concluded that soil nitrification can be circumvented without penalty, although physical differences in the resulting plants were apparent when compared to controls. This is not meant to underestimate the importance of nitrification since a large portion of the nitrogen added to soils, either as fertilizer or through microbial transformations, is eventually oxidized to nitrate (74). The suggestion, rather, is that nitrogen fixation is ultimately the more important process although some substitution in the form of industrial and geologic fixation is possible.

The agents of nitrogen fixation are either bacteria or blue-green algae (75). Generally when nitrogen fixation by other organisms is reported, further investigation (76) reveals the presence of nitrogen fixing bacteria in the gut. Biologic nitrogen fixation may be symbiotic

or nonsymbiotic. Symbiotic nitrogen fixation results from a mutualistic relationship between a leguminous plant and a bacterium of the genus Rhizobium. Although it was thought that these bacteria could not fix nitrogen when separated from the plant, preliminary evidence (77) indicates that at least one strain of Rhizobium can fix nitrogen on certain laboratory media.

The most important agents of nonsymbiotic nitrogen fixation are the heterocystic blue-green algae, Anabaena and Nostoc, and the bacteria, Azotobacter and Beijerinckia (70). This ability is widespread among the blue-green algae, occuring in Aphanizomenon, Calothrix, and Trichodesmium (78), and the bacteria, being reported in the genera Bacillus, Clostridium, Desulfovibrio (79), Klebsiella (80), Rhodospirillum, Chromatium, and Pseudomonas (11). There have also been reports (81) of genetic transfers of nitrogen-fixing ability to organisms such as E. coli. Thus, there are a wide variety of species from which to choose a representative of nitrogen-fixation.

(3) Sulfur Cycle

Sulfur is widely distributed in nature occurring as iron pyrites, galena, sphalerite, cinnabar, stibnite, gypsum and epsom salts as well as other forms (82). It is incorporated into cellular metabolism primarily in the form of sulfate by microorganisms and plants, and the resulting reduced sulfur compounds, such as sulfur-containing amino acids, can be further metabolized by microorganisms, plants and animals. The decomposition of this material to H_2S can be carried out by many groups of microorganisms such as Desulfovibrio. The oxidation of H_2S to elemental sulfur and thence to sulfate may be bacterial or spontaneous (70). Members of the photosynthetic families, Chromatiaceae and Chlorobiaceae, oxidize H_2S to elemental sulfur, while the family Beggiatoaceae and the genus Thiobacillus oxidize sulfur to sulfate.

The sulfur cycle is further complicated by chemicals such as dimethyl sulfide, whose role in balancing this cycle is not completely understood (83). Natural forces, such as volcanic activity, also contribute to this cycle (84). Man's activities in the form of increased industrial production of reduced sulfur compounds have an effect on the sulfur balance (85). Severe environmental problems, such as the formation of acid mine water, can result when the sulfur cycle is altered. However, since the reduced sulfur formed as a result of metabolic processes or industrial pollution can be spontaneously oxidized to provide more sulfate, the cycle is not absolutely dependent on microbial interconversions as is the nitrogen cycle. Since the interconversions of sulfate are part of the normal cellular metabolic processes, no special organisms need to be considered.

(4) Phosphorus Cycle

Phosphorus is found in nature mainly in the form of phosphate, predominantly as orthophosphate, organic phosphate esters or the mineral apatite (86). Generally, the main agents of cyclic transfer in the phosphorus cycle are physical processes such as precipitation and solubilization. Precipitation removes phosphate by forming insoluble calcium and iron salts. Phosphate is added to the system by solubilization of these salts from rocks. Microorganisms may participate in this "cycle" in several ways, but they are not the main agents. For example, while they may use the available phosphorus in an area, they cannot replenish the supply to any great extent. In some instances, microorganisms may resolubilize precipitated phosphorus by acid production. They may also decompose phosphorus compounds through the action of extracellular enzymes such as deoxyribonucleases, ATP ases and phosphatases (11). However, physical processes are mainly responsible for the available phosphate supply. Therefore, although phosphorus

may be a limiting nutrient in the environment, its availability in a metabolically usable form, inorganic orthophosphate, is generally not dependent on microbial interactions (86, 87). Since biological interconversions of phosphate can be studied as part of the normal cellular metabolism, no special organisms need to be selected.

c. Candidate Organisms Based on Vital Processes

These considerations of microorganisms as indicators of the continued functioning of vital processes have emphasized three areas. One is the general metabolism of carbon compounds to carbon dioxide and water with the concomitent generation of energy through the Tricarboxylic Acid Cycle and the Electron Transport System. Second is the process of photosynthesis which provides for the continued production of oxygen and organic matter through the fixation of carbon dioxide into cellular material. Third is the process of nitrogen fixation in which the inert gas, N_2, is transformed to ammonia, a biologically usable form.

Representative microorganisms capable of carrying out each process have been listed in Table 2. To avoid excessive length, the genera are not listed separately if the particular process is characteristic of an order or a family. For example, the genus Pseudomonas appears under Process 1 as the family Pseudomonadaceae because all the members are capable of respiratory metabolism but as the genus Pseudomonas under Process 3 since not all genera in the family have been reported to be capable of fixing nitrogen.

Members of several genera are good subjects for studying more than one of these processes. The blue-green algae (Cyanophyta) are good examples because they are nitrogen-fixing, photosynthetic organisms. They are also capable of heterotrophic growth in the presence of air, although the mechanism differs from the Tricarboxylic Acid Cycle and

BIOSPHERICS INCORPORATED

Table 2

Representative Microorganisms Exhibiting
Selected Vital Processes

	Process	Organisms
1.	Metabolism Via Tricarboxylic Acid Cycle and Electron Transport System	Actinomyces (Some Species) Arthrobacter Azotobacteriaceae Bacillaceae Corynebacterium Enterobacteriaceae Micrococcaceae Myxobacterales Neisseriaceae Pseudomonadaceae Rhizobiaceae Vibrionaceae
2.	Photosynthesis	Chlorophyta Chrysophyta Pyrrophyta Cyanophyta Chlorobiaceae Chromatiaceae Rhodospirillaceae
3.	Nitrogen Fixation	Cyanophyta Achromobacter Aerobacter Azotobacter Bacillus Beijerinckia Chlorobium Chromatium Clostridium Desulfovibrio Klebsiella Methanobacter Pseudomonas Rhizobium Rhodopseudomonas Rhodospirillum

BIOSPHERICS INCORPORATED

Electron Transport Systems. All the photosynthetic bacteria can fix nitrogen. Most of these bacteria are obligate anaerobes, but even those that can grow in the presence of air (Rhodospirillum) do so only in the dark and demonstrate slow growth rates. Many of the nonphotosynthetic nitrogen-fixing bacteria do metabolize by these pathways, namely, Achromobacter, Aerobacter, Azotobacter, Bacillus, Berijerinckia, and Pseudomonas. Although each process can be studied in any of the organisms listed in Table 2 as having that ability, those in which more than one process can be studied will be emphasized. A more definitive choice depends upon the ecosystem(s) in which these processes are to be studied.

Other considerations are involved in the final choice of the candidates. The selection must result in a system/organism that can survive the trip to its point of use either in a resting stage, in a cryptobiotic form, lyophilized, frozen, or in a dynamic situation. Table 3 lists those organisms which are known to form resting stages or undergo crytobiosis, permitting their surval under adverse conditions (88, 89, 90). It should be noted that this includes many of the organisms under considera tion.

C. Choice of Candidate Ecosystems

In summary, the model ecosystem(s) to be used as a Basic Warning System will consist of at least two parts (Table 4). In one, the conditions will be maximized for the survival of Martian organisms, thus providing the best chance for them to multiply and demonstrate an effect on the metabolism of a terrestrial organism. In the other, terrestrial conditions suitable for studying the selected vital terrestrial processes will be maintained. However, to the extent the terrestrial organisms will permit, the Martian organism will not be exposed to conditions extreme to the Mars environment. For example, a saline environment will be avoided, if possible, because of the presumed absence of large amounts of water and salt on Mars.

BIOSPHERICS INCORPORATED

Table 3

Survival Stages of Various Organisms

Organism	Survival Stage
Actinomycetales	Spore
Azotobacter	Cyst
Bacillaceae	Spore
Cyanophyta	Akinete
Rhodomicrobium	Spore
Sporocytophaga	Microcyst
Rotifera	Cryptobiosis
Tardigrada	Cryptobiosis
Amoeba	Cyst

BIOSPHERICS INCORPORATED

Table 4

Candidate Ecosystems

A. Martian Conditions System: The metabolism of a hardy terrestrial organism will be challenged by the Mars sample under Mars-like conditions.

B. Vital Process System: Representatives of vital processes will be challenged by the Mars sample in one of the following arrangements:

Single 1. Nitrogen-fixing blue-green alga in which metabolism is alternated between photosynthesis and heterotrophy by environmental control.

2. Lichen in which algal photosynthesis provides oxygen for fungal metabolism which produces carbon dioxide for the alga. Alga must fix nitrogen.

3. Algal-Bacterial Association whereby algal photosynthesis provides oxygen for bacterial heterotrophy which produces carbon dioxide for the alga. One member should fix nitrogen.

Dual 4. Alga and Nitrogen-Fixing Aerobic Bacterium. Photosynthesis is tested in the alga. Tricarboxylic Acid metabolism, respiration and nitrogen-fixation are tested in a nitrogen-fixing aerobic heterotrophic bacterium (bacterium common to Processes 1 and 3, Table 2).

5. Bacterium (Process 1, Table 2) and Nitrogen-Fixing Blue-Green Alga. Tricarboxylic Acid metabolism and respiration are tested in bacterium selected from list of aerobic heterotrophs in Table 2. Photosynthesis and nitrogen-fixation tested in blue-green alga.

These two approaches have several advantages when used in conjunction. As discussed previously, the Martian conditions give the Martian organism the best opportunity to multiply and demonstrate any adverse effects, while the terrestrial conditions permit observations of these effects on key processes. Additionally, comparison of these two conditions may provide information on the inhibitory effects of the terrestrial environment. Finally, these approaches expose the Martian organisms to several types of environmental conditions, several terrestrial organisms and several different vital processes.

Possible terrestrial candidates for the first approach, the Martian ecosystem, have been listed in Table 1. Those organisms which will be studied first, as a result of the discussion in Section II. B. 2. d., are the green algae Chlorella, the blue-green algae Nostoc, the photosynthetic bacterium Rhodospirillum, the heterotrophic bacterial genera, Actinomyces, and Corynebacterium, and the lichen Nephroma. Of these organisms, those able to metabolize under the most extreme conditions need to be determined in the laboratory. Particularly, the minimal levels of moisture at which these organisms can metabolize needs to be established. It is possible that one of the vital processes may be studied in the Martian test system (see below).

In the second approach, we are looking for one or more ecosystems in which the vital processes of aerobic heterotrophic respiration, photosynthesis and nitrogen-fixation can be tested. Once the best system or combination of systems has been determined, specific organisms can be selected from those listed in Table 2. Obviously, if several processes can be tested in one organism, the number of test systems can be reduced.

Generally, photosynthetic organisms do not possess the entire Tricarboxylic Acid Cycle and do not respire by means of the Tricarboxylic Acid Cycle and the Electron Transport System. Although photorespiration

has been observed (64), it occurs at a low rate and does not truly represent aerobic respiration. Five types of simple terrestrial systems that permit observation of the three processes are listed in Table 4.

The first system consists of a nitrogen-fixing blue-green alga in which metabolism is alternated between photosynthesis and heterotrophic metabolism by environmental control. Although algal metabolism has been cycled in the laboratory, it is suggested that this system not be chosen because a one-component system minimizes exposure of terrestrial organisms to the Martian organism. Further, if all three processes are to function, the environment must cycle between two sets of conditions. It is well known that multiple stresses are synergistic in effect, and this system would subject the Martian organism to unnecessary stresses. It is important that the growth of this organism not be inhibited. Moreover, this is a complex system in that the terrestrial organism must switch back and forth between two opposing types of metabolism and stabilize at the same measurable level each time if we are to be able to determine that a variance in metabolism was caused by the Martian organism. Finally, although these organisms are growing heterotrophically in the air, functioning of the entire Tricarboxylic Acid Cycle and Electron Transport System cannot be observed in these organisms. For these reasons, it is suggested that this system not be considered any further.

The second system consists of a lichen in which algal photosynthesis provides oxygen for fungal metabolism which, in turn, produces carbon dioxide for the alga. The alga provides nitrates for the fungus and itself by fixing molecular nitrogen into ammonia and oxidizing it to nitrates. However, before lichens can be utilized, several problems must be overcome. First, lichens characteristically metabolize at a slow rate. This may make monitoring difficult and an effect on metabolism of the Martian organism may not be readily

discernable. Second, it is difficult to maintain lichens in the laboratory (70). Under favorable growth conditions, lichens dissociate into free-living algae and fungi. Stressed conditions such as a low nutrient level, combined with alternating periods of wetting and drying will stabilize the symbiosis. However, this would subject the Martian organism to additional, and cyclic, stresses. Finally, lichens produce acids believed to inhibit other microorganisms. These may be inhibitory to the Martian forms. Several of these problems, such as whether the metabolic rate is too low for short-term monitoring, can only be resolved by laboratory tests. On the other hand, lichens have many advantages as test systems. Using this system, all three vital processes can be studied in a nitrogen-fixing lichen. Also, more than one organisms would be exposed to the Martian organism. Finally, lichens are known to grow under conditions of cold and low nutrients. Because of these advantages, the problems posed by the lichen system should be evaluated in laboratory studies.

The third system consists of an algal-bacterial association in which algal photosynthesis provides oxygen for bacterial heterotrophy which, in turn, produces carbon dioxide for the alga. One member of this association would be specifically selected to fix nitrogen. This system possesses several advantages. First, more than one organism is exposed to the Martian sample. Second, the rate of metabolism can be controlled at a level high enough that small variances will be apparent. Third, as opposed to the two previous systems, no cyclic environmental stresses are necessary. This will materially simplify the engineering design and required support system. Fourth, a balanced two-component system is highly sensitive because of the absolute interdependencies of the components. However, this sensitivity also makes it difficult to maintain. Such a system will require laboratory testing to determine whether the balance between the two organisms can be maintained for the necessary length of time.

BIOSPHERICS INCORPORATED

Systems Four and Five can be considered together since they are basically the same. Both systems are dual systems containing the same two components, a blue-green alga and a bacterium. The systems differ in whether nitrogen-fixation is studied in the bacterial or algal component. There are many advantages to a dual system. First, as in Systems Two and Three, several terrestrial microorganisms are exposed to the Martian forms. Second, no cyclic environmental stresses need be applied to the Martian organism. Third, conditions for each terrestrial organism can be controlled separately permitting one to be tested under Martian conditions. Fourth, changes in the rate of metabolism will provide a sensitive monitor. These systems should also be laboratory tested.

Thus, following the elimination of System One, in which environmental conditions must be cycled so that the organism would alternate between photosynthesis and heterotrophic metabolism, four microbial systems remain for testing in the laboratory. These consist specifically of (1) a lichen, (2) an algal bacterial symbiosis, (3) an alga and a nitrogen-fixing aerobic bacterium, and (4) an aerobic heterotrophic bacterium and a nitrogen-fixing blue-green algae.

BIOSPHERICS INCORPORATED

III. PRELIMINARY DEFINITION STUDY OF PLANETARY RECEIVING LABORATORY (PRL)

A. Summary of Quarterly Activities

The following activities were carried out in conjunction with our study of the requirements of a Planetary Receiving Laboratory:

- A tour of the storage facilities for lunar material at Johnson Spaceflight Center and discussions with knowledgeable NASA personnel connected with the Lunar Receiving Laboratory were conducted.
- The quarantine protocol documents for the lunar program were reviewed in conjunction with the above discussions.
- A NASA Johnson Spaceflight Center survey on the impact of sterilization regimens on petrological, geochemical and geophysical characterization of a returned planetary sample was reviewed.
- A questionnaire and cover letter to be used in a survey of potential Planetary Return sample experiments was formulated.
- The compilation of a mailing list of scientists potentially interested in a Mars Return Sample was initiated.

B. Problems Encountered in the Lunar Return Sample Program

The tour and discussions with NASA personnel experienced in the Lunar Return Sample Program confirmed our preliminary conclusions (91):

- The biological containment systems are inadequate.
- Much of the physical instrumentation is incompatible with biological barrier concepts in use.
- Better quarantine protocols and experiment design are needed to maximize the use of the return sample. In

particular, specialized equipment requiring small samples and compatible with quarantine requirements are needed.

- Much of the quarantine protocol was poorly defined. For example, it was said to be harder to get material from the primary storage chamber to an analytical instrument inside the Lunar Receiving Laboratory than it was to take this same material outside to a separate facility.

- Use of small, resealable containers which can be easily sterilized would offer major advantages over the glove box systems.

In addition, Dr. M. Reynolds, officer for sample contamination in the lunar program, and Dr. M. Duke, Curator of Lunar Materials, summarized their recommendations for a Planetary Return Sample Program based on observations of the problems encountered in the Lunar Program:

- An overview panel of scientists should be established and should exercise strong advisory control over all phases of the program, including, particularly, the planning, design, protocol formulation, and sample utilization prioritization.

- A contamination control group should be established in the planning phase of the program and should provide advice to the design engineers. This control group should follow the program to completion and be charged with the responsibility and given sufficient authority to insure that contamination of the sample is held to a minimum in the acquisition, experimentation, and storage phases of the program.

- It should be recognized that competition for samples will be very strong and an equitable and scientifically sound method and machinery for allocating samples should be instituted as early in the program as practicable.

- The sample requirements for life detection and biohazard assessment were large in the Lunar Program. Development of techniques for minimizing the sample requirements for these functions should receive high priority.

- Strong management control of the interrelated functions of preservation, experimentation, and quarantine maintenance should be instituted for a Planetary Return Sample Mission.

Dr. Kelton Ferguson and others of Johnson Spaceflight Center also contributed views on the Lunar Program and a possible planetary mission. The views did not depart from the above summary in any significant way.

C. Development of Questionnaire

In the process of preparing our questionnaire for soliciting information about potential Planetary Return Sample experiments, we reviewed the results of a Johnson Spaceflight Center survey on the effects of sample sterilization. The responses were useful in identifying scientists in the physical sciences with a high degree of interest in a Planetary Return Sample Mission and in formulating the present questionnaire.

A draft of our proposed questionnaire is attached as Appendix I for NASA review. The cover letter is written for Dr. Richard S. Young's signature although the logistics of printing; mailing and processing the responses would be performed by Biospherics Incorporated personnel. It was felt that an official NASA inquiry under Dr. Young's name would be more effective in soliciting responses.

BIOSPHERICS INCORPORATED

IV. FUTURE PLANS

During the next reporting period, the varying methods of transporting and maintaining candidate organisms for long periods will be surveyed and their restrictions considered in selecting the organisms to be tested. Preliminary laboratory efforts will be directed toward the acquisition of these strains and establishment of culture maintenance techniques. Since the moisture level is an important consideration in both approaches to ecosystem development, the minimal moisture level that will permit active metabolism, and not merely survival, will be determined for the strains under consideration.

In regard to the design of the Planetary Receiving Laboratory, the questionnaire will be modified to reflect NASA's comments and will be sent to scientists and engineers for completion. The list of interested scientists is being compiled from experimenters in the lunar and Viking programs and recognized experts in the life and physical sciences who are thought to have an interest in a Mars Sample Return Mission. The returns from the survey will be categorized and a preliminary assessment of the results will also be completed in the upcoming period.

Respectfully submitted,

Jayne F. Carney

Jayne F. Carney, Ph. D.
Research Microbiologist

J. Michael Hall

J. Michael Hall
Systems Engineer

Gilbert V. Levin

Gilbert V. Levin, Ph. D.
Principal Investigator

BIOSPHERICS INCORPORATED

REFERENCES

1. Mars Surface Sample Return Mission Workshop, NASA, Washington, D.C., June 11-12 (1974).

2. Phillips, G.P., "Back Contamination," in Planetary Quarantine, Ed. L. Hall, Gordon and Breach Pub. Co., New York (1971).

3. Space Science Board Conference on Potential Hazards of Back Contamination from the Planets, National Academy of Science, National Research Council, Washington, D.C. (1965).

4. The Role of Lunar Receiving Laboratory in Post-Apollo Biological and Biomedical Activities, NAS9-9728, American Institute of Biological Sciences, Washington, D.C. (1970).

5. Lederberg, J., "Exobiology: Approaches to Life Beyond the Earth," Science, 132, 393 (1960).

6. Levinthal, E.C. and Lederberg, J., "Relationship of Planetary Quarantine to Biological Search Strategy," in Life Sciences and Space Research VI, Ed. A.H. Brown and F.G. Favorite, North-Holland Publ. Co., Amsterdam (1968).

7. Alexander, M., "Possible Contamination of Earth by Lunar or Martian Life," Nature, 222, 432 (1969).

8. "Automated Microbial Metabolism Laboratory," Annual Report for Contract No. NASW-2280, Prepared for NASA Headquarters, Biospherics Incorporated, Washington, D.C. (1973).

9. Young, R.S. and DeVincenzi, D., "From Mars With Love," Science, 186, 495 (1974).

10. Klein, H., "On the Viking Search for Life on Mars," J. Astronom. Soc. of the Pacific, Interview (1974).

11. Doetsch, R.N. and Cook, T.M., Introduction to Bacteria and their Ecobiology, University Park Press, Baltimore (1973).

12. Cooke, G.D., Beyers, R.J. and Odum, E.P., "The Case for the Multispecies Ecological System, with Special Reference to Succession and Stability," Bioregenerative Systems, NASA SP 165, National Aeronautics and Space Administration, Washington, D.C. (1966).

BIOSPHERICS INCORPORATED

13. Hanel, R., Conrath, B., Hovis, W., Kunde, V., Lowman, P., Maguire, W., Pearl, J., Pirroglea, J., Prabhakara, C., Schlachman, B., Levin, G., Straat, P. and Burke, T., "Investigation of the Martian Environment by Infrared Spectroscopy of Mariner 9," Icarus, 17, 423 (1972).

14. Hartman, W.K. and Raper, O., The New Mars, The Discoveries of Mariner 9, NASA, Washington, D.C. (1974).

15. Balsamo, S.R. and Salisbury, J.W., "Slope Angle and Frost Formation on Mars, Icarus, 18, 156 (1973).

16. Siegel, S.M., Renwick, G., Daly, O., Guimarro, C., Davis, G. and Halpern, L., "The Survival Capabilities and the Performance of Earth Organisms in Simulated Extraterrestrial Environments," in Current Aspects of Exobiology, Ed., G. Mamikunian and M.H. Briggs, California Institute of Technology, California (1965).

17. Imshenetskiy, A.A., Abyzov, S.S., Voronov, G.T., Zhukova, A.I. and Lysenko, S.V., "The Possibility of Life In Outer Space," in Life Sciences and Space Research IV, Ed. A.H. Brown and M. Florkin, Spartan Books, Washington, D.C. (1966).

18. Imshenetskiy, A.A., and Lysenko, S.V., "Effect of High Vacuum on Microorganisms," in Extraterrestrial Life and Its Detection Methods, Ed., A.A. Imshenetskiy, Nauka Press, Moscow (1970).

19. Moseley, E.B., "The Repair of Damaged DNA in Irradiated Bacteria," in Advances in Microbial Physiology, Ed., A.H. Rose and J.F. Wilkinson, Academic Press, New York (1968).

20. Koller, L.R., Ultraviolet Radiation, John Wiley and Sons, New York (1965).

21. Phillips, G.B. and Handel, E., Jr., Use of Ultraviolet Radiation in Microbial Laboratories, BL Technical Report 28, U.S. Army Biological Laboratories, Fort Detrick, Md. (1965).

22. Aerobiology, American Association for the Advancement of Science, Publication 17, Washington, D.C. (1942).

BIOSPHERICS INCORPORATED

23. Kirensky, L.V., Terskov, I.A., Gitelson, I.I., Lisovsky, G.M., Kovrov, B.G. and Okladnikov, Y.N., "Experimental Biological Life Support System," in Life Sciences and Space Research VI, Ed., A.H. Brown and F.G. Favorite, North-Holland Publ. Co., Amsterdam (1968).

24. Walker, J.D. and Colwell, R.R., "Enumeration of Petroleum Degrading Microorganisms," Appl. Microbiol. (In Press).

25. Litchfield, C.D., Rake, J.B., Zindulis, J., Watanabe, R.T. and Stein, D.J., "Optimization of Procedures for the Recovery of Heterotrophic Bacteria from Marine Sediments," Microbial Ecol., 1, 219 (1975).

26. Scher, S., Packer, E. and Sagan, C., "Biological Contamination of Mars," in Life Sciences and Space Research III, Ed. J. Florkin, John Wiley and Sons, Inc., New York (1965).

27. Roberts, T.L. and Wynne, E.S., Studies With A Simulated Martian Environment, TDR 62-121, USAFSAM, Brooks Air Force Base, Texas (1962).

28. Roberts, T.L., Ball, R.J. and Wynne, E.S., Studies With A Simulated Martian Environment, TDR 62-151, USAFSAM, Brooks Air Force Base, Texas (1963).

29. Hawrylewicz, E.J., Hagan, C.A. and Ehrlich, R., "Response of Microorganisms to a Simulated Martian Environment," in Life Sciences and Space Research III, Ed., M. Florkin, John Wiley and Sons, Inc., New York (1965).

30. Hawrylewicz, E.J., Hagan, C.A. and Ehrlich, R., "Survival and Growth of Potential Microbial Contaminants in Space Environments," in Life Sciences and Space Research IV, Ed., A.H. Brown and M. Florkin, Spartan Books, Washington, D.C. (1966).

31. Hawrylewicz, E.J., Hagan, C.A. and Ehrlich, R., "Response of Microorganisms to a Simulated Martian Environment," in Life Sciences and Space Research III, Ed., M. Florkin, North-Holland Publ. Co., Amsterdam (1965).

32. Young, R.S., Deal, P.H. and Whitfield, O., "Response of Soil Bacteria to High Temperatures and Diurnal Freezing and Thawing," Nature, 216, 355 (1967).

33. Young, R.S., Deal, P.H. and Whitfield, O., "The Response of Spore Forming Vs. Nonspore-Forming Bacteria to Diurnal Freezing and Thawing," Space Life Sciences, 1, 113 (1968).

34. Young, R.S., Deal, P.H., Bell, J. and Allen, J.L., "Bacteria Under Simulated Martian Conditions," in Life Sciences and Space Research II, Ed., M. Florkin and A. Dollus, North-Holland Publ. Co., Amsterdam (1964).

35. Young, R.S., Deal, P.H., Bell, J. and Allen, J.L., "Effect of Diurnal Freeze-Thawing on Survival and Growth of Selected Bacteria," Nature, 199, 1078 (1963).

36. Bychenkova, V.N. and Lozina-Lozinsky, L.K., "Resistance of Infusoria to Low Pressure, Anoxia and Intense Cooling," in Extraterrestrial Life and Its Detection Methods, Ed., A.A. Imshenetskiy, Nauka Press, Moscow (1970).

37. Lozina-Lozinsky, L.K., "Resistance of Organisms to Extreme Influences in Relation to Some Exobiological Problems," in Life Sciences and Space Research VI, Ed., A.H. Brown and F.G. Favorite, North-Holland Publ. Co., Amsterdam (1968).

38. Fahey, B.D., "An Analysis of Diurnal Freeze-Thaw and Frost Heave Cycles in the Indian Peaks Region of the Colorado Front Range," Arctic and Alpine Res., 5, 269 (1973).

39. Miller, P.C., Collier, B.D. and Bunnell, F., "Development of Ecosystem Modeling in the U.S. IBP Tundra Biome," Proc. Systems Analysis in Ecology Symposium, Vol. 3, Athens, Georgia (1973).

40. Coyne, P.I. and Kelley, J.J., "Release of CO_2 from Frozen Soil to the Arctic Atmosphere," Nature, 234, 407 (1971).

41. Clarholm, M., Lid-Torsvik, V. and Baker, J.H., "Bacteria Populations of Some Fennoscandian Tundra Soils," in Fennoscandian Tundra Ecosystems," Ed., F.E. Wielgolaski, Springer-Verlag, Berlin (1975).

42. Parinkina, O.M., "Determination of Bacterial Growth Rates in Tundra Soils," in Modern Methods in the Study of Microbial Ecology, Ed., T. Rosswall, NFR, Stockholm (1973).

43. Boyd, W.L. and Boyd, J.W., "Microorganisms in Frost Scars," Arctic and Alpine Res., 4, 257 (1972).

44. U.S. Participation in the International Biological Program, Report No. 6 of the U.S. National Committee, National Academy of Sciences, Washington, D.C. (1974).

45. Gersper, P.L. and Arkley, R.J., "Soil Nutrients," in U.S. IBP-Tundra Biome Report 70-1, Ed., J. Brown and G.C. Wert (1970).

46. Alexander, V. and Schell, D.M., "Seasonal and Spatial Variation of Nitrogen Fixation in the Barrow, Alaska Tundra," Arctic and Alpine Res., 5 (1973).

47. Kallio, P., "Ecology of Nitrogen Fixation in Subarctic Lichens," Oikos, 25, 194 (1974).

48. Kallio, S. and Kallio, P., "Nitrogen Fixation in Lichens at Kevo, North-Finland," in Fennoscandian Tundra Ecosystems, Ed., F.E. Weilgolaski, Springer-Verlag, Berlin (1975).

49. Grandhall, V. and Lid-Torsvik, V., "Nitrogen Fixation by Bacteria and Free Living Blue-Green Algae in Tundra Areas," in Fennoscandian Tundra Ecosystems, Ed., F.E. Weilgolaski, Springer-Verlag, Berlin (1975).

50. Tieszen, L.L., "Photosynthesis and Respiration in Arctic Tundra Grasses," Arctic and Alpine Res., 5, 239 (1973).

51. Hoham, R.W., "Optimum Temperatures and Temperature Ranges for Growth of Snow Algae," Arctic and Alpine Res., 7, 13 (1975).

52. Chapin, F.S., VanCleve, K. and Tieszen, L.L., "Seasonal Nutrient Dynamics of Tundra Vegetation at Barrow, Alaska," Arctic and Alpine Res., 7, 109 (1975).

53. Cloudsley-Thompson, J.L., Terrestrial Environments, John Wiley and Sons, Inc., New York (1975).

54. Cameron, R.E. and Blank, G.B., "Soil Studies - Microflora of Desert Regions," Space Programs Summary IV, Jet Propulsion Laboratory, Pasadena, California (1965).

55. Alexander, M., Introduction to Soil Microbiology, John Wiley and Sons, Inc., New York (1961).

BIOSPHERICS INCORPORATED

56. Cameron, R.E. and Blank, G.B., Desert Algae: Soil Crusts and Diaphanous Substrata as Algal Habitats, Technical Report No. 32-971, Jet Propulsion Laboratory, Pasadena, California (1966).

57. Rice, C.W., Uydess, I.L., Hempfling, W.P. and Vishniac, W.V., "Isolation of Microorganisms from Soils of the Antarctic Dry Valleys," Presented, Annual Meeting of American Society for Microbiology, New York City (1975).

58. Horowitz, N.H., Cameron, R.E. and Hubbard, J.S., "Microbiology of the Dry Valleys of Antarctica," Science, 176, 242 (1972).

59. Cameron, R.E., Morelli, A. and Johnson, R.M., "Bacteria Species in Soil and Air in the Antarctic Continent," Antarctic J. U.S., 7, 187 (1972).

60. Hempfling, W.P., "Microorganisms Indigenous to the Antarctic Dry Valleys," Report (NGR 33 109 002) to National Aeronautics and Space Administration, Washington, D.C. (1975).

61. Glasstone, S., The Book of Mars, SP-179, National Aeronautics and Space Administration, Washington, D.C. (1968).

62. Costello, D.F., The Desert World, T.Y. Crowell Co., New York, New York (1972).

63. Beschel, R.E., "Lichens as a Measure of the Age of Recent Moraines," Arctic and Alpine Res., 5, 303 (1973).

64. Lehninger, A.L., Biochemistry, Worth Publ., Inc., New York (1975).

65. Mahler, H.R. and Cordes, E.H., Biological Chemistry, Harper and Row, New York (1966).

66. Levin, G.V., "Detection of Metabolically Produced Labeled Gas: The Viking Mars Lander, Icarus, 16, 153 (1972).

67. Forrest, W.W. and Walker, D.J., "The Generation and Utilization of Energy During Growth," in Advances in Microbial Physiology, Ed., A.H. Rose and J.F. Wilkinson, Academic Press, New York (1971).

68. Bergey's Manual of Determinative Bacteriology, 8th Edition, Ed., R.E. Buchanan and N.E. Gibson, Williams and Wilkins Co., Baltimore, Maryland (1974).

69. Guarria, L.J., "Interrelationship of the Major Nutrients and Micro-organisms," in The Aquatic Environment, Ed., L.J. Guarria and R.K. Ballentine, Environmental Protection Agency, Washington, D.C. (1972).

70. The Microbial World, Ed., R.Y. Stanier, M. Doudoroff and E.A. Adelberg, Prentice-Hall, Inc., New Jersey (1970).

71. Vishniac, W., "Extraterrestrial Microbiology," Aerospace Med., 31, 678 (1960).

72. Lascelles, J., "The Bacterial Photosynthetic Apparatus," in Advances in Microbial Physiology, Ed., A.H. Rose and J.F. Wilkinson, Academic Press, New York (1968).

73. Hardy, R.W.F. and Holsten, R.D., "Global Nitrogen Cycling," in The Aquatic Environment, Ed., L.J. Guarria and R.K. Ballentine, Environmental Protection Agency, Washington, D.C. (1972).

74. National Academy of Sciences, Accumulation of Nitrate, National Academy of Sciences, Washington, D.C. (1972).

75. Benemann, J.R. and Valentine, R.C., "The Pathways of Nitrogen Fixation in Advances in Microbial Physiology, Ed., A.H. Rose and D.W. Tempest, Academic Press, New York (1972).

76. Carpenter, E.J. and Culliney, J.L., "Nitrogen Fixation in Marine Shipworms," Science, 187, 551 (1975).

77. McComb, J.A., Elliott, J. and Dilworth, M.J., "Acetylene Reduction by Rhizobium in Pure Culture," Nature, 256, 409 (1975).

78. Stewart, W.D.P., "Nitrogen Fixation by Photosynthetic Microorganisms," Ann. Rev. Microbiol., 27, 283 (1973).

79. Jones, K., "Nitrogen Fixation in a Salt Marsh," J. Ecol., 62, 553 (1974).

BIOSPHERICS INCORPORATED

80. Werner, D., Evans, H.J. and Seidler, R.J., "Facultatively Anaerobic Nitrogen-Fixing Bacteria from the Marine Environment," Can. J. Microbiol., 20, 59 (1974).

81. Dixon, R.O. and Postgate, J.R., "Transfer of Nitrogen Fixation Genes by Conjugation in Klebsiella pneumonia," Nature, 234, 47 (1971).

82. Handbook of Chemistry and Physics, Ed., R.C. Weast, CRC Press, Cleveland, Ohio (1973).

83. Lovelock, J.E., Maggs, R.J. and Rasmussen, R.A., "Atmospheric Dimethyl Sulfide and the Natural Sulfur Cycle," Nature, 237, 452 (1972).

84. LeGall, J., "The Sulfur Cycle," in The Aquatic Environment, Ed., L.J. Guarria and R.K. Ballentine, Environmental Protection Agency, Washington, D.C. (1972).

85. Kellog, W.W., Cadle, R.D., Allen, E.R., Lazaues, A.L. and Martell, E.A., "The Sulfur Cycle," Science, 175, 587, (1972).

86. Brock, T.D., Principles of Microbial Ecology, Prentice-Hall, Inc., New Jersey (1966).

87. Fuhs, G.W., "Microbial Influences in Phosphorus Cycling," in The Aquatic Environment, Ed., L.J. Guarria and R.K. Ballentine, Environmental Protection Agency, Washington, D.C. (1972).

88. Sudo, S.Z. and Dworkin, M., "Comparative Biology of Procaryotic Resting Cells," in Advances in Microbiol. Physiology, Ed., A.H. Rose and D.W. Tempest, Academic Press, New York (1973).

89. Crowe, J.H. and Cooper, A.F., "Cryptobiosis," Scientific American, 225, 30 (1971).

90. Griffiths, A.J., "Encystment in Amoeba," in Advances in Microbial Physiology, Ed., A.H. Rose and J.F. Wilkinson, Academic Press, New York (1970).

91. "Technology for Return of Planetary Samples," Annual Report for Contract No. NASW-2280, Prepared for NASA Headquarters, Biospherics Incorporated, Washington, D.C. (1975).

APPENDIX I

Dear _____:

NASA is considering the feasibility of returning material from the Martian surface in the 1980's. As a part of the overall feasibility analysis, it is necessary to define, at least in broad terms, the kinds of experiments to be performed on the returned material, the availability of suitable instrumention, and the experimental difficulties which might arise from sterilization procedures and quarantine constraints. The objective of the attached questionnaire is to obtain such information. We are not addressing here the larger issues of whether a Martian sample should be returned or what, if any, sterilization procedure would be prudent.

A principal use of the questionnaire data will be in evaluating the feasibility of quarantine protocols, the facilities and design of a Planetary Receiving Laboratory, and overall approaches to experimentation on a returned sample. Many of you have had direct experience at a detailed and experiment specific level on the Lunar program. NASA believes that this valuable experience should be utilized in planning for future planetary missions. Recognizing that you have a busy schedule, we have attempted to design the questionnaire to minimize the time required for you to provide the information.

Please complete the questionnaire giving the specified information for what you believe to be the most significant experiment in your field of expertise. If you feel other experiments are also of prime importance, please complete one questionnaire for each experiment. Additional forms are available from this office or you can duplicate the enclosed form.

A number of the questions are directed at determining the feasibility of sample sharing and the minimum sample size required for each experiment. Since only a very small quantity of sample can be returned, this issue is very important. It is also imperative that needs for special equipment necessary for a valid experiment under quarantine conditions be identified early. This information can provide a basis for establishing priorities and needs for equipment development.

Other concise information, in the form of written comments, research abstracts, papers, etc., which you feel could aid in understanding the problems to be encountered in a Planetary Return Sample Experimentation Program would be appreciated.

The information will be used only in the development of Planetary Return Sample objectives. A Planetary Return Sample Project has not yet been authorized by NASA. This is not a Request for Proposal.

We request that you fill out the attached questionnaire and return it by _____.

Thank you very much for your cooperation.

PLANETARY RETURN SAMPLE QUESTIONNAIRE

1. Descriptive title of experiment:

   Scientist (Name of Scientist Responding to Questionnaire. List co-investigators if appropriate).

   a. Name:

   b. Organization Affiliation:

   c. Address:

   d. Telephone:

2. Brief description of experiment:

3. Scientific justification of experiment:

4. Parameters to be measured:

5. Is your experiment dependent upon other experiments or analyses performed on the sample? _____ If so, which?

6. Briefly describe equipment required (type, sensitivity, accuracy, etc.):

7. Is commercial equipment available? If so, list type and identifying nomenclature for equipment which requires minimum quantity sample:

8. How many discrete samples are required?

9. What is the minimum size and form for each sample?

10. Does your experimental technique alter the sample? _____. If so, describe cause and alterations expected:

11. Describe environmental conditions required during sample storage which are critical to validity of your experiment:

12. Describe environmental conditions required during the experiment:

13. List other experiments which could be performed on your sample prior to your experiment:

14. List other experiments which could be performed on your sample after your experiment:

15. List key contaminants and concentrations which would seriously interfere with your experiment:

16. It is anticipated that some form of heat sterilization might be required. Define the adverse effects of the following sterilization regimens on your experiment:

a. 110°C, dry, Martian atmosphere, cyclical from 20° to 40°C, 24-hour cycle for 90 days:

Effect: No__; Minor__ ; Severe__ ; Unknown ___

Comment on affected parameters:

b. 150°C, dry, Martian atmosphere, 30 days:

Effect: No__ ; Minor __; Severe_ Unknown __

Comment on affected parameters:

c. 150°C, dry, Martian atmosphere, one week:

Effect: No__ ; Minor __; Severe__ ; Unknown __

Comment on affected parameters:

d. 350°C, dry, Martian atmosphere, one day

Effect: No __; Minor __; Severe __; Unknown

Comment on affected parameters:

17. Could your experiment be performed within an isolation chamber using remote manipulators? _____. Comment briefly (estimate size and capability required of chamber and manipulator, i.e., special equipment):

18. Could your experiment be performed by an astronaut scientist _____ or technician _____ under your remote supervision? Comment briefly:

19. It may be necessary to perform your experiment under very strict quarantine conditions. Comment on the degree of access absolutely essential to the success of your experiment, manipulation techniques applicable and difficulties you foresee in operating under limited access conditions and for post-experiment cleanup: